89 Hypermutation in *Burkholderia cepacia* complex is highly prevalent in chronic respiratory infections of cystic fibrosis patients

A. Bosch[1], P. Martina[2], S. Feliziani[3], C. Juan[4], M. Bettiol[5], B. Gatti[6], B. Valeiras[7], O. Yantorno[7], A. Smania[8], A. Oliver[4]. *[1]CINDEFI-CONICET-CCT La Plata, Universidad Nacional de La Plata, Chemistry, La Plata, Argentina; [2]CINDEFI-UNLP-CONICET-CCT La Plata, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Chemistry, La Plata, Argentina; [3]CIQUIBIC-CONICET Centro de Investigaciones en Química Biológica de Córdoba, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Cordoba, Argentina; [4]Servicio de Microbiología y Unidad de Investigación, Hospital Universitario Son Espases, Palma de Mallorca, Spain, Palma de Mallorca, Spain; [5]Sala de Microbiología, Hospital de Niños de La Plata 'Sor María Ludovica', Microbiología, La Plata, Argentina; [6]Sala de Microbiología, Hospital de Niños de La Plata 'Sor María Ludovica', La Plata, Argentina; [7]CINDEFI-UNLP-CONICET-CCT La Plata, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina; [8]CIQUIBIC-CONICET Centro de Investigaciones en Química Biológica de Córdoba, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina*

Aims: The aims of this work was to describe the occurrence of hypermutable strains in *Burkholderia cepacia* complex (Bcc) species recovered in Argentina, and to analyse the sequence of *mutS* and *mutL* genes, which were reported to be the most commonly affected genes leading to hypermutabilty in other species. In addition we analysed whether antimicrobial resistance is related to hypermutation.
Methods: Spontaneous mutation rates were determined by resistance to Rifampicin in 125 Bcc isolates recovered from 100 CF patients, 10 non-CF patients and 15 environmental samples. Eight primers were designed to span the *mutS* and *mutL* genes and the corresponding sequences were analyzed. Antimicrobial resistance against 17 antibiotics was determined for the whole population by Vitek automated sensitivity cards.
Results: An overall prevalence of 13.6% (17 isolates) of hypermutator isolates in the analyzed population was encountered. Out of the 27 CF chronically infected patients studied, 40.7% were colonized by hypermutable strains. The sequence analysis of *mutS* and *mutL* genes showed that 13 isolates (76.5%) were defective in these two main components of the mismatch repair system. The antimicrobial resistance study performed revealed high rates of resistance among isolates from CF patients for all the Bcc species. Nevertheless, no significant association was found between increased antibiotic resistance and hypermutation with an exception for ciprofloxacin.
Conclusion: The high prevalence of Bcc hypermutators isolates among chronically colonized CF patients suggest that hypermutaton might be playing a key role in increasing bacterial adaptability and persistence in Bcc chronic infection.

90 *Burkholderia contaminans* in cystic fibrosis: genetic and phenotypic diversity among isolates from long-term infections

A. Bosch[1], P. Martina[1], C. Prieto[2], B. Valeiras[1], M. Bettiol[3], P. Montanaro[4], O. Yantorno[1]. *[1]CINDEFI-UNLP-CONICET-CCT La Plata, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Chemistry, La Plata, Argentina; [2]CINDEFI-UNLP-CONICET-CCT La Plata, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina; [3]Sala de Microbiología, Hospital de Niños de La Plata "Sor María Ludovica", La Plata, Argentina; [4]Hospital Santísima Trinidad de Córdoba, Laboratorio de Microbiología, Córdoba, Argentina*

Aim: The aim of the present work was to get insights into the genetic and phenotypic diversity of *B. contaminans* clinical isolates recovered from long-term infection in cystic fibrosis (CF) patients.
Methods: A total of 65 *B. contaminans* isolates recovered from serial sputum cultures from 13 chronically infected patients attended at 3 CF treatment centers in Argentina during a 10-year period (2004–2013) was analyzed. Isolates were identified by *recA*-PCR sequencing and *recA*-RFLP-HaeIII restriction analysis, and genotyping by BOX-PCR fingerprinting. Phenotypic diversity among the isolates was analyzed, by colony morphology, growth rate, production of haemolysin, lipases and proteases expression, EPS and biofilm formation, antibiotic resistance and by Fourier transform infrared (FT-IR) spectroscopy.
Results: The whole *B. contaminans* population 11 displayed different BOX subtypes. Nine BOX-subtypes were found during the first year of infection for the different patients. Nevertheless, the genotypic diversity of the isolates decreased for all the isolates retrieved from chronically infected patients, where only 2 Box subtypes could be determined. In contrast to this genotyping diversity evolution, a high phenotypic diversity – with a decrease expression of the virulence factors – characterized the isolates recovered both from different patients and from each patient along the chronic infections.
Conclusion: Our results revealed evidences of genetic and phenotypic adaptation of *B. contaminans* isolates during chronic lung infections that could contribute to increase the capacity of these bacteria to survive and persist in their host.

91 Epidemiological study of CF patients with chronic *Pseudomonas aeruginosa* colonization in a reference center in Brazil

N.C. Caçador[1], C.P.C. Capizzani[1], L.A.G.M.M. Torres[2], M.I.M. Fernandes[2], R. Martinez[3], L. Tonani[1], L.A. Pereira[3], A.L.C. Darini[1]. *[1]Faculdade de Ciências Farmacêuticas de Ribeirão Preto – Universidade de São Paulo, Ribeirão Preto, Brazil; [2]Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto – Universidade de São Paulo, Departamento de Puericultura e Pediatria, Ribeirão Preto, Brazil; [3]Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto – Universidade de São Paulo, Laboratório de Microbiologia, Ribeirão Preto, Brazil*

P. aeruginosa (PA) is a leading cause of morbidity in CF. This study evaluated PA in patients with chronic colonization, concerning antimicrobial susceptibility, resistance genes (RG), virulence factors (VF), genomic similarity and clinical data. From 2011 to 2012, isolates were identified by biochemical tests and PCR; antibiogram was performed; VF and genes encoding resistance to beta-lactams and quinolone were investigated by PCR and macrorestriction profiles were analyzed by *Spe*I-PFGE. From 101 patients, colonization was found in approximately 24% of patients under 13 years old and 52% of older individuals. PFGE analysis revealed 21 pulsotypes, 5 of them were detected in more than one patient. The virulence genes *algD*, *exoS*, *exoY*, *lasI*, *lasR*, *rhlI*, *lasB, plcH* and *plcN* were observed in more than 85% of the isolates, whereas genes *rhlR*, *phzM, phzS, apr* were observed in less than 75%. Nevertheless, no correlation was observed between genes and clinical severity, as they presented the same frequency in patients with higher or lower impairment. Lung function was below average: in patients under 13, FVC was 1.8 L and FEV1 1.4 L, in older patients FVC = 2.8 L and FEV1 = 1.8 L. Exacerbations were similar in both groups (2/year) and BMI was adequate. However, Shwachmann score was slightly altered in the older group. Resistance profiles were higher for levofloxacin (26%), imipenem and ciprofloxacin (21%). The isolates with these resistance profiles were investigated for the presence of RG, but none were found. This could explain the good clinical evolution of this group with preservation of nutritional status. PFGE revealed that interpatient transmission of strains may have occurred.

92 Molecular epidemiological surveillance of *Pseudomonas aeruginosa* at a Swedish CF centre over a >10-year period (1999−2012)

E.A. Johansson[1,2], A. Lindblad[3,4], M. Gilljam[4,5,6], C. Welinder-Olsson[1,2]. *[1]Sahlgrenska University Hospital, Clinical Microbiology, Gothenburg, Sweden; [2]Gothenburg University, Inst of Biomedicine, Gothenburg, Sweden; [3]Sahlgrenska University Hospital/East, Queen Silvia Childrens Hospital, Gothenburg, Sweden; [4]Gothenburg CF Centre, Sahlgrenska University Hospital, Gothenburg, Sweden; [5]Sahlgrenska University Hospital, Dep of Respiratory Medicine and Allergology, Gothenburg, Sweden; [6]Gothenburg University, Inst of Medicine, Gothenburg, Sweden*

Objectives: Cystic fibrosis (CF) patients are frequently colonized with *Pseudomonas aeruginosa* (*Pa*). Reports of cross-infection at CF Centres have led to recommendations of segregation. In Sweden instead strict hygienic routines were applied in the outpatient clinic. In order to survey the prevalence and the incidence of patient transmission of *P.a* at the CF centre in Gothenburg we regularly perform genotyping of *P.a* isolates.
Methods: From 1999 until 2012 *P.a* isolates were genotyped using PFGE and the results were confirmed by MLVA and compared with genotypes of all available *P.a* isolates obtained routinely at our laboratory during the study period.
Results: 104 patients attending Gothenburg CF centre, provided 232 *P.a* isolates for genotyping during the period. We identified 78 unique genotypes among the analysed isolates of which 56 genotypes (72%) were isolated from single patients. The environmental B-strains were shared by 13 patients and the earlier identified camp transmission clone J-strains were isolated from 8 patients. The diversity observed with PFGE was in 91% agreement with the diversity found by MLVA analysis. Of the CF patients included, 42% were determined as chronically colonized between the early 1990s and 2004. Thereafter 14 patients of the remaining 59 intermittent *P.a* infected became chronically colonized, none with the frequent B-clone.
Conclusions: The *P.a* recovered from Gothenburg CF centre patients showed high genotypic diversity and no sign of cross infection concluding that the hygienic routines seemed to have prevented frequent patient-to-patient transmission at the centre until 2012.